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(54) Title: METHOD OF COMBATING UNDESIRABLE HYPERPLASTIC CELL PROLIFERATION

(57) Abstract: A method of combating undesirable cell proliferation in a patient the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or a prodrug thereof. A method of preventing neointimal hyperplasia in a patient the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or a prodrug thereof.

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METHOD OF COMBATING UNDESIRABLE HYPERPLASTIC CELL PROLIFERATION

The present invention relates to a new use for low molecular weight thrombin inhibitors such as melagatran and its derivatives, and for prodrugs thereof.

International Patent Application WO 94/29336 discloses a group of compounds that are useful as inhibitors of serine proteases, such as thrombin and/or kininogenases. The thrombin-inhibiting compounds are thus indicated as anticoagulants, and the kininogenase-inhibiting compounds as anti-inflammatory agents.

One of the thrombin-inhibiting compounds that is specifically disclosed in WO 94/29336 is HOOC-CH_2-(*R*)-Cgl-Aze-Pab-H which is also known as melagatran (see Example 1 of WO 94/29336, and the list of abbreviations in that document). The use of low molecular weight thrombin inhibitors such as melagatran and its derivatives for treating undesirable hyperplastic cell proliferation has not been disclosed previously.

Recurrence of ischemic symptoms due to vessel renarrowing remains the major long-term limitation to successful treatment of coronary artery disease. About 10-40% of patients undergoing any kind of revascularization procedure, such as PTCA (percutaneous transluminal coronary angioplasty), CABG (coronary artery bypass graft) or coronary or peripheral grafting will have to undergo a second procedure within 6 months due to vessel renarrowing. Although a variety of pharmaceutical agents have been tested in clinical trials most of them have proven ineffective at reducing the rate of vessel narrowing.

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We have now shown that a low molecular weight thrombin inhibitor can inhibit hyperplastic cell proliferation and, surprisingly, that this effect is seen in cellular systems where cells are stimulated to proliferate in the absence of added thrombin.

A first aspect of the invention provides a method of combating undesirable hyperplastic cell proliferation in a patient the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or a prodrug thereof.

By "combating undesirable hyperplastic cell proliferation" is included both the treatment and the prevention of undesirable cell proliferation. Hyperplasia is the abnormal multiplication or increase in the number of normal cells in normal arrangement in a tissue.

By "undesirable hyperplastic cell proliferation" is included any undesirable hyperplastic cell proliferation in the human body, particularly that which may lead to pathologic conditions.

It is preferred if the undesirable cell proliferation to be combated is undesirable proliferation of smooth muscle cells. Undesirable proliferation of smooth muscle cells is one component in intimal hyperplasia. Thus, the methods and uses of the invention may be useful in treating intimal hyperplasia. It is particularly preferred if the undesirable cell proliferation to be combated is the undesirable cell proliferation of vascular smooth muscle cells. The proliferation of vascular smooth muscle cells is a key event in the development of intimal hyperplasia. Restenosis can be considered to be due to neointimal hyperplasia ie an abnormal increase in the number of normal cells in a normal arrangement in the newly formed inner layer of the blood vessels. Smooth muscle cells not only proliferate,

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but they will also migrate from their normal position in the media into the intimal layer of the vessel wall.

For the avoidance of doubt, intimal hyperplasia, in the context of the invention, includes restenosis (ie a new stenosis which occurs after the primary stenosis has been removed mechanically (eg by PTCA)). In addition, a surgical intervention, such as graft implantation, often occludes at the site of the junctions. In this case there is no primary stenosis which is responsible for the occlusion: this is also intimal hyperplasia.

Thus, a further aspect of the invention provides a method of preventing intimal hyperplasia in a patient, the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or prodrug thereof. The method of the invention is useful in preventing restenosis.

Further aspects of the invention provide use of a low molecular weight thrombin inhibitor or a prodrug thereof in the manufacture of a medicament for combating undesirable cell proliferation in a patient and use of a low molecular weight thrombin inhibitor or a prodrug thereof in the manufacture of a medicament for preventing intimal hyperplasia.

The term "low molecular weight thrombin inhibitor" will be understood by those skilled in the art. The term may also by understood to include any composition of matter (eg chemical compound) which inhibits thrombin to an experimentally determinable degree in *in vivo* and/or in *in vitro* tests, and which possesses a molecular weight of below 2,000, preferably below 1,000.

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Preferred low molecular weight thrombin inhibitors include low molecular weight peptide, and amino acid, based thrombin inhibitors and/or peptide analogue, based thrombin inhibitors.

Thus, the term "low molecular weight thrombin inhibitors" includes thrombin inhibitors with one to four peptide linkages, and/or with a molecular weight below 1000, and includes those described in the review paper by Claesson in *Blood Coagul. Fibrin.* (1994) **5**, 411, as well as those disclosed in US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 95/23609, WO 93/05069, WO 97/46577, WO 98/01422, WO 95/35309, WO 96/25426, WO 94/29336, WO 93/18060, WO 95/01168, WO 97/23499 and WO 97/02284; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596, the disclosures in all of which documents are hereby incorporated by reference.

Preferred low molecular weight peptide-based thrombin inhibitors include those known collectively as the "gatrans". Particular gatrans which may be mentioned include HOOC-CH_2-(*R*)Cha-Pic-Nag-H (known as inogatran; see International Patent Application WO 93/11152 and the list of abbreviations therein) and HOOC-CH_2-(*R*)Cgl-Aze-Pab-H (known as melagatran; see International Patent Application WO 94/29336 and the list of abbreviations therein) and prodrugs thereof (see eg WO 97/23499). Particularly preferred thrombin inhibitors include melagatran.

Low molecular weight thrombin inhibitors include any pharmaceutically acceptable derivatives of the inhibitors described above.

"Pharmaceutically acceptable derivatives" includes salts (eg pharmaceutically acceptable non-toxic organic or inorganic acid addition salts) and solvates.

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The low molecular weight thrombin inhibitors for use in the methods of the invention, including melagatran, and derivatives and prodrugs thereof, may be administered for systemic delivery to the site of undesirable cell proliferation, or may be administered for delivery directly (locally) to that site, using appropriate means of administration that are known to the skilled person. It is believed that a combination of an acute intravenous infusion during the intervention and a prolonged oral prophylaxis might be the optimal treatment.

Thus, in accordance with the invention, the low molecular weight thrombin inhibitor such as melagatran, and derivatives and prodrugs thereof, may be administered orally, intravenously, subcutaneously, buccally, rectally, dermally, nasally, tracheally, bronchially, topically, by any other parenteral route, or *via* inhalation, in the form of a pharmaceutical preparation comprising the active ingredient in a pharmaceutically acceptable dosage form. Depending on the disorder, and the patient, to be treated, as well as the route of administration, the compositions may be administered at varying doses.

Preferred modes of delivery are systemic. For melagatran and derivatives thereof, preferred modes of administration are parenteral, more preferably intravenous, and especially subcutaneous. For prodrugs of melagatran, preferred modes of administration are oral.

In the therapeutic treatment of mammals, and especially humans, the low molecular weight thrombin inhibitors and prodrugs thereof may be administered alone, but will generally be administered as a pharmaceutical formulation in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, which may be selected with due regard to the intended route of administration and standard pharmaceutical practice. The

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preparation of suitable formulations for use in administering low molecular weight thrombin inhibitors such as melagatran, derivatives and prodrugs thereof is described in the literature, for example as described in *inter alia* International Patent Applications WO 94/29336, WO 96/14084, WO 96/16671, WO 97/23499, WO 97/39770, WO 97/45138 and WO 98/16252, the disclosures in which documents are hereby incorporated by reference. Otherwise, the preparation of suitable formulations may be achieved non-inventively by the skilled person using routine techniques.

Suitable formulations for use in administering thrombin inhibitors are known in the art, and include those known from US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 93/18060, WO 93/05069, WO 94/20467, WO 94/29336, WO 95/35309, WO 95/23609, WO 96/03374, WO 96/06832, WO 96/06849, WO 96/25426, WO 96/32110, WO 97/01338, WO 97/02284, WO 97/15190, WO 97/30708, WO 97/40024, WO 97/46577, WO 98/06740, WO 97/49404, WO 97/11693, WO 97/24135, WO 97/47299, WO 98/01422 and WO 98/57932; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642, 669 317, 601 459 and 623 596, the disclosures in all of which documents are hereby incorporated by reference.

The amount of low molecular weight thrombin inhibitor or prodrug thereof, in the formulation will depend on the severity of the condition, and on the patient, to be treated, as well as the compound(s) which is/are employed, but may be determined non-inventively by the skilled person.

According to a further aspect of the invention there is provided a pharmaceutical formulation for use in combating undesirable cell proliferation or for use in preventing neointimal hyperplasia comprising an effective amount of a low molecular weight thrombin inhibitor or a

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pharmaceutically acceptable derivative or prodrug thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Low molecular weight thrombin inhibitors and prodrugs thereof, may also be combined with other therapeutic agents that are useful in combating undesirable cell proliferation and/or other therapeutic agents that are useful in the treatment of a disease characterised by undesirable cell proliferation as one of its symptoms. When low molecular weight thrombin inhibitors and prodrugs thereof, are "combined" with other therapeutic agents in this way, the active ingredients may be administered together in the same formulation, or administered separately (simultaneously or sequentially) in different formulations. Heparin, and platelet aggregation inhibitors (GPIIb IIIa inhibitors) are presently used to prevent acute vessel occlusion.

Similarly, low molecular weight thrombin inhibitors may be "combined" with prodrugs of low molecular weight inhibitors, the inhibitor and prodrug being administered together in the same formulation, or administered separately (simultaneously or sequentially) in different formulations.

Suitable doses of low molecular weight thrombin inhibitor such as melagatran, prodrugs and derivatives thereof, in the therapeutic and/or prophylactic treatment of mammalian, especially human, patients are typically those which give a mean plasma concentration in the range 0.001 to 5 μmol/L. In any event, the physician, or the skilled person, will be able to determine the actual dosage which will be most suitable for an individual patient, which is likely to vary with the age, weight, sex and response of the particular patient. The above concentrations are exemplary of the average case; there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

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The skilled person will also be appreciated that the low molecular weight thrombin inhibitor or prodrug thereof, may be administered in an appropriate dose on an "as required" basis (ie as needed or desired).

The term "prodrug" of a low molecular weight thrombin inhibitor includes any compound that, following oral or parenteral administration, is metabolised *in vivo* to form a low molecular weight thrombin inhibitor (as defined herein), in an experimentally-detectable amount, and within a predetermined time (eg within a dosing interval of between 6 and 24 hours (ie once to four times daily)), following oral or parenteral administration. Prodrugs of the thrombin inhibitor melagatran that may be mentioned include those disclosed in international patent application WO 97/23499. Preferred prodrugs are those of the formula R^1O_2C-CH_2-(*R*)Cgl-Aze-Pab-OH (see the list of abbreviations in WO 97/23499), wherein R^1 represents benzyl or linear or branched C_{1-6} alkyl (eg C_{1-4} alkyl, especially methyl, propyl and, particularly, ethyl) and the OH group replaces one of the amidino hydrogens in Pab.

The invention will now be described in more detail by reference to the following Figures and non-limiting Examples wherein:

Figure 1 shows the inhibition of serum stimulated human smooth muscle cells by melagatran.

Figure 2 shows the inhibition of thrombin stimulated human smooth muscle cell proliferation by melagatran. The concentration that inhibited cell proliferation by 50% (IC_{50}) was calculated to be 100±79 (n=5) and 150 ±132 nmol/L (n=3) for arterial and venous smooth muscle cells, respectively.

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Example 1: Effect of melagatran on proliferation of serum-stimulated smooth muscle cells

Materials

Complete medium: Waymouth medium 12-738 (Bio Wittaker, USA) supplemented with 10% fetal bovine serum (FBS, PAA Laboratories Gmbh, Germany), 60 U/ml Penicillin-Streptomycin, 1.2 mM L-glutamine (Sigma, USA) and 2 mM sodium pyruvate (Bio Wittaker, USA).

Minimal medium: As above but with 0.5 % FBS instead of 10%.

96-well Tissue Culture Test Plates (Techno Plastic-Products AG, Schweiz), test compounds synthesized at AstraZeneca (Mölndal, Sweden), BrdU-kit (Boehringer Mannheim, Germany, Cat no 1647229), SpectraMAX PLUS microplate reader (Molecular Devices, USA).

The technique used to study cell proliferation is based on the incorporation of a thymidine analogue (5-bromo-2'-deoxyuridine, BrdU) into the DNA of proliferating cells. After incorporation into the DNA, BrdU is detected by immunoassay using monoclonal antibodies labelled with peroxidase (POD). A cell proliferation ELISA BrdU kit from Boehringer Mannheim was used and the procedure outlined in the supplier's manual was followed.

Methods (serum stimulated cell proliferation)

Smooth muscle cells were isolated from human material, either from *vena saphena magna* or *arteria uterina*, according to the method by Fager *et al*, *Exp. Cell. Res.* **176**, 319-35 (1988), as described below.

1. Trypsinize and count the cells (if you are using a Coulter Counter, use 12.4 µm as lower size of the cells). Add 100 µl and 2-10 x10^3 cells/well in complete medium and allow the cells to attach 24-48 h.

Example 1: Effect of melagatran on proliferation of serum-stimulated smooth muscle cells

Materials

Complete medium: Waymouth medium 12-738 (Bio Wittaker, USA) supplemented with 10% fetal bovine serum (FBS, PAA Laboratories Gmbh, Germany), 60 U/ml Penicillin-Streptomycin, 1.2 mM L-glutamine (Sigma, USA) and 2 mM sodium pyruvate (Bio Wittaker, USA).

Minimal medium: As above but with 0.5 % FBS instead of 10%.

96-well Tissue Culture Test Plates (Techno Plastic-Products AG, Schweiz), test compounds synthesized at AstraZeneca (Mölndal, Sweden), BrdU-kit (Boehringer Mannheim, Germany, Cat no 1647229), SpectraMAX PLUS microplate reader (Molecular Devices, USA).

The technique used to study cell proliferation is based on the incorporation of a thymidine analogue (5-bromo-2'-deoxyuridine, BrdU) into the DNA of proliferating cells. After incorporation into the DNA, BrdU is detected by immunoassay using monoclonal antibodies labelled with peroxidase (POD). A cell proliferation ELISA BrdU kit from Boehringer Mannheim was used and the procedure outlined in the supplier's manual was followed.

Methods (serum stimulated cell proliferation)

Smooth muscle cells were isolated from human material, either from *vena saphena magna* or *arteria uterina*, according to the method by Fager *et al*, *Exp. Cell. Res.* **176**, 319-35 (1988), as described below.

1. Trypsinize and count the cells (if you are using a Coulter Counter, use 12.4 μm as lower size of the cells). Add 100 μl and 2-10 $x10^3$ cells/well in complete medium and allow the cells to attach 24-48 h.

Add just medium instead of cells to one set of wells as blank. Use SMC's in passage 5-10.

2. Remove the medium by inverting the plate following tapping on absorbent tissue. Wash one time with PBS. Add 100 µl minimal medium. Incubate for 24-60 h.

3. Replace again with 90 µl complete medium and add directly 10 µl of the compounds to be tested or the vehicle to a control set of wells. Add minimal medium to one set of wells as a control for non-proliferating cells. Incubate for 24-48 h.

4. Add 10 µl BrdU labelling solution to all wells except one set for a background control, and reincubate for 4-6 h. BrdU from the kit should be diluted 1:100 with minimal medium (for one plate prepare 1 ml).

5. Remove labelling medium by inverting the plate following tapping on absorbent tissue. Add 200 µl FixDenat (makes the cell membrane permeable to the BrdU antibody, see next step) and incubate at room temperature for 30 min.

6. Remove FixDenat by inverting the plate following tapping on absorbent tissue. Add 100 µl anti-BrdU-POD and incubate at room temperature for 30-120 min. A stock solution of freeze-dried anti-BrdU-POD was prepared by dilution in 1.1 ml redistilled water and stored in 100 µl aliquots at –20°C. The anti-BrdU-POD stock solution was further diluted 1:100 immediately before use.

7. Remove antibody conjugate by inverting the plate. Rinse wells three times with 200-300 µl washing solution (PBS).

Add just medium instead of cells to one set of wells as blank. Use SMC's in passage 5-10.

2. Remove the medium by inverting the plate following tapping on absorbent tissue. Wash one time with PBS. Add 100 µl minimal medium. Incubate for 24-60 h.

3. Replace again with 90 µl complete medium and add directly 10 µl of the compounds to be tested or the vehicle to a control set of wells. Add minimal medium to one set of wells as a control for non-proliferating cells. Incubate for 24-48 h.

4. Add 10 µl BrdU labelling solution to all wells except one set for a background control, and reincubate for 4-6 h. BrdU from the kit should be diluted 1:100 with minimal medium (for one plate prepare 1 ml).

5. Remove labelling medium by inverting the plate following tapping on absorbent tissue. Add 200 µl FixDenat (makes the cell membrane permeable to the BrdU antibody, see next step) and incubate at room temperature for 30 min.

6. Remove FixDenat by inverting the plate following tapping on absorbent tissue. Add 100 µl anti-BrdU-POD and incubate at room temperature for 30-120 min. A stock solution of freeze-dried anti-BrdU-POD was prepared by dilution in 1.1 ml redistilled water and stored in 100 µl aliquots at –20°C. The anti-BrdU-POD stock solution was further diluted 1:100 immediately before use.

7. Remove antibody conjugate by inverting the plate. Rinse wells three times with 200-300 µl washing solution (PBS).

8. Remove washing solution by inverting the plate following tapping on absorbent tissue. Add 100 µl substrate solution (tetramethylbenzidine) and incubate at room temperature until colour development is sufficient for photometric detection (5-60 min).

9. Measure the absorbency of the samples in the microplate reader at 370 nm (reference wavelength: 492 nm).

Results

Inhibition of serum-induced proliferation of smooth muscle cells isolated from the human *vena saphena magna* is illustrated in Figure 1. After subtraction of the proliferation rate of non-stimulated cells (0.5% fetal calf serum), melagatran was found to inhibit cell proliferation in a dose dependent way with a maximum of 60% inhibition at a concentration of 1 µmol/L.

Example 2: Effect of melagatran on cell proliferation of thrombin-stimulated smooth muscle cells

Methods (thrombin stimulated cell proliferation)

Experiments with thrombin as inducer of cell proliferation were carried out as described in Example 1 but with the following modifications.

Human thrombin was obtained from Haematologic Technologies Inc.

Minimal medium: Fetal calf serum was exchanged for 0. 1 % human albumin.

After step 2 in Example 1 above, two extra steps have been introduced:

8. Remove washing solution by inverting the plate following tapping on absorbent tissue. Add 100 µl substrate solution (tetramethylbenzidine) and incubate at room temperature until colour development is sufficient for photometric detection (5-60 min).

9. Measure the absorbency of the samples in the microplate reader at 370 nm (reference wavelength: 492 nm).

Results

Inhibition of serum-induced proliferation of smooth muscle cells isolated from the human *vena saphena magna* is illustrated in Figure 1. After subtraction of the proliferation rate of non-stimulated cells (0.5% fetal calf serum), melagatran was found to inhibit cell proliferation in a dose dependent way with a maximum of 60% inhibition at a concentration of 1 µmol/L.

Example 2: Effect of melagatran on cell proliferation of thrombin-stimulated smooth muscle cells

Methods (thrombin stimulated cell proliferation)

Experiments with thrombin as inducer of cell proliferation were carried out as described in Example 1 but with the following modifications.

Human thrombin was obtained from Haematologic Technologies Inc.

Minimal medium: Fetal calf serum was exchanged for 0. 1 % human albumin.

After step 2 in Example 1 above, two extra steps have been introduced:

3a. Replace again with 80 µl minimal medium and add directly 10 µl of thrombin (2 units/ml) to all wells except one set with minimal medium as a control for non-proliferating cells. Add directly 10µl of the test compounds or just minimal medium to the controls and to one set measuring the thrombin effect in the absence of inhibitors. Incubate for 48 h.

3b. Repeat stimulation/inhibition as in 3a and incubate for 28-32 h.

4. Add BrdU and reincubate for 16-20 h.

Results

Inhibition of thrombin induced proliferation of smooth muscle cells was performed on cells isolated from either from *vena saphena magna or arteria uterine,* according to the method by Fager *et al, Exp. Cell. Res.* **176,** 319-35 (1988). Melagatran was in these experiments, in contrast to the case with serum stimulated cells, able to completely inhibit proliferation of smooth muscle cells. An example of a dose-response curve is illustrated in Figure 2. The concentration that inhibited cell proliferation by 50% (IC_{50}) was calculated to be 100±79 (n=5) and 150 ±132 nmol/L (n=3) for arterial and venous smooth muscle cells, respectively.

Example 3: Effect of melagatran on vascular smooth muscle cells in rats

The antiproliferative effect of melagatran on vascular smooth muscle cells was studied in male Sprague-Dawley rats. After anaesthesia, a balloon catheter was inserted into the right common carotid artery. Endothelial denudation was achieved by mechanically pulling a balloon catheter inflated at 3 atm pressure 5 times within a distance of 1 to 1.5 cm in the vessel lumen. The rats were given melagatran as an intravenous bolus dose of 4 µmol/kg immediately after denudation of the endothelium and then as a

3a. Replace again with 80 µl minimal medium and add directly 10 µl of thrombin (2 units/ml) to all wells except one set with minimal medium as a control for non-proliferating cells. Add directly 10µl of the test compounds or just minimal medium to the controls and to one set measuring the thrombin effect in the absence of inhibitors. Incubate for 48 h.

3b. Repeat stimulation/inhibition as in 3a and incubate for 28-32 h.

4. Add BrdU and reincubate for 16-20 h.

Results

Inhibition of thrombin induced proliferation of smooth muscle cells was performed on cells isolated from either from *vena saphena magna or arteria uterine,* according to the method by Fager *et al*, *Exp. Cell. Res.* **176**, 319-35 (1988). Melagatran was in these experiments, in contrast to the case with serum stimulated cells, able to completely inhibit proliferation of smooth muscle cells. An example of a dose-response curve is illustrated in Figure 2. The concentration that inhibited cell proliferation by 50% (IC_{50}) was calculated to be 100±79 (n=5) and 150 ±132 nmol/L (n=3) for arterial and venous smooth muscle cells, respectively.

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continuous intravenous infusion from a peristaltic pump placed under the skin on the neck. The infusion rate was 1.5 μmol/kg per hour and continued for 14 days. Rat infused with saline served as controls. At the end of the experiment the rats were anaesthetised with foren and the abdominal part of the aorta was uncovered. A catheter was then inserted into the aorta and the vessels of the rat were perfused with saline until the perfusate was almost free of blood and the rats were dead. The vessels were then perfused with 4% formaldehyde at 110 mm Hg pressure and at a speed of 20 ml/min. The right common carotid artery was isolated and immersed in 4% formaldehyde, dehydrated and finally embedded in paraffin. The artery in the paraffin block was then sliced (≈ 5 μm thick) and cross-sections were mounted on glass slides and stained with hematoxylin. The area of the intimal hyperplasia (neointima) was then measured under light microscopy connected to a computer.

Results

The neointima area (μm^2) from all rats are summarised in the table below.

Melagatran infused rats	Saline infused rats
72491	208072
182469	104540
63889	100355
121597	70315
88151	74591
126017	88922
169235	35184
94259	205521
Mean 114764	**128423**
SEM# 15369	**22252**

#Standard Error of Mean

continuous intravenous infusion from a peristaltic pump placed under the skin on the neck. The infusion rate was 1.5 μmol/kg per hour and continued for 14 days. Rat infused with saline served as controls. At the end of the experiment the rats were anaesthetised with foren and the abdominal part of the aorta was uncovered. A catheter was then inserted into the aorta and the vessels of the rat were perfused with saline until the perfusate was almost free of blood and the rats were dead. The vessels were then perfused with 4% formaldehyde at 110 mm Hg pressure and at a speed of 20 ml/min. The right common carotid artery was isolated and immersed in 4% formaldehyde, dehydrated and finally embedded in paraffin. The artery in the paraffin block was then sliced (≈ 5 μm thick) and cross-sections were mounted on glass slides and stained with hematoxylin. The area of the intimal hyperplasia (neointima) was then measured under light microscopy connected to a computer.

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94259	205521
Mean 114764	**128423**
SEM# 15369	**22252**

#Standard Error of Mean

The mean plasma concentration of melagatran immediately after the bolus dose was 3.8 μmol/L and during the infusion 2.5 μmol/L (day 7) and 1.7 μmol/L (day 14). The size of the neointima, caused by endothelial denudation of the right common carotid artery, was 11% smaller in melagatran treated rats compared to the neointima in control rats infused with saline.

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CLAIMS

1. A method of combating undesirable hyperplastic cell proliferation in a patient the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or a prodrug thereof.

2. A method according to Claim 1 wherein the undesirable cell proliferation is undesirable proliferation of smooth muscle cells.

3. A method according to Claim 2 wherein the undesirable cell proliferation is undesirable proliferation of vascular smooth muscle cells.

4. A method of preventing intimal hyperplasia in a patient the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or a prodrug thereof.

5. A method according to any one of the preceding claims wherein the low molecular weight thrombin inhibitor is melagatran or a derivative thereof, or a prodrug of either.

6. Use of a low molecular weight thrombin inhibitor or a prodrug thereof in the manufacture of a medicament for combating undesirable cell proliferation in a patient.

7. Use of a low molecular weight thrombin inhibitor or a prodrug thereof in the manufacture of a medicament for preventing intimal hyperplasmia.

8. Use according to Claim 6 or Claim 7 wherein the undesirable cell proliferation is as defined in any one of Claims 2 or 3.

CLAIMS

1. A method of combating undesirable hyperplastic cell proliferation in a patient the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or a prodrug thereof.

2. A method according to Claim 1 wherein the undesirable cell proliferation is undesirable proliferation of smooth muscle cells.

3. A method according to Claim 2 wherein the undesirable cell proliferation is undesirable proliferation of vascular smooth muscle cells.

4. A method of preventing intimal hyperplasia in a patient the method comprising administering to the patient an effective amount of a low molecular weight thrombin inhibitor or a prodrug thereof.

5. A method according to any one of the preceding claims wherein the low molecular weight thrombin inhibitor is melagatran or a derivative thereof, or a prodrug of either.

6. Use of a low molecular weight thrombin inhibitor or a prodrug thereof in the manufacture of a medicament for combating undesirable cell proliferation in a patient.

7. Use of a low molecular weight thrombin inhibitor or a prodrug thereof in the manufacture of a medicament for preventing intimal hyperplasmia.

8. Use according to Claim 6 or Claim 7 wherein the undesirable cell proliferation is as defined in any one of Claims 2 or 3.

9. Use according to any one of Claims 6 to 8 wherein the low molecular weight thrombin inhibitor is melagatran or a derivative thereof, or a prodrug of either.

10. A pharmaceutical composition for use in combating undesirable cell proliferation in a patient or for use in preventing neointimal hyperplasia in a patient comprising a low molecular weight thrombin inhibitor, or a prodrug thereof, and a pharmaceutically acceptable carrier.

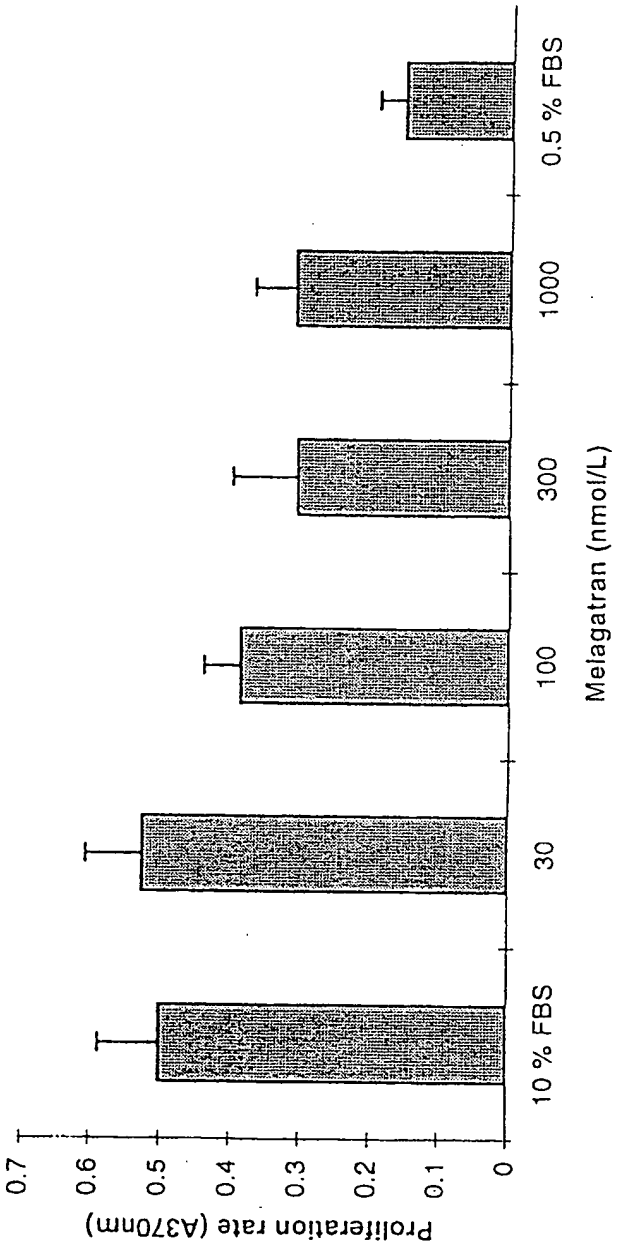
11. A method or use as claimed in Claim 5 or Claim 9 (as appropriate) wherein the prodrug of melagatran is of the formula R^1O_2C-CH_2-(*R*)Cgl-Aze-Pab-OH, wherein R^1 represents benzyl or linear or branched C_{1-6} alkyl.

9. Use according to any one of Claims 6 to 8 wherein the low molecular weight thrombin inhibitor is melagatran or a derivative thereof, or a prodrug of either.

10. A pharmaceutical composition for use in combating undesirable cell proliferation in a patient or for use in preventing neointimal hyperplasia in a patient comprising a low molecular weight thrombin inhibitor, or a prodrug thereof, and a pharmaceutically acceptable carrier.

11. A method or use as claimed in Claim 5 or Claim 9 (as appropriate) wherein the prodrug of melagatran is of the formula R^1O_2C-CH_2-(*R*)Cgl-Aze-Pab-OH, wherein R^1 represents benzyl or linear or branched C_{1-6} alkyl.

Figure 1


Proliferation rate (A370nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10 % FBS
30
100
300
1000
0.5 % FBS
Melagatran (nmol/L)

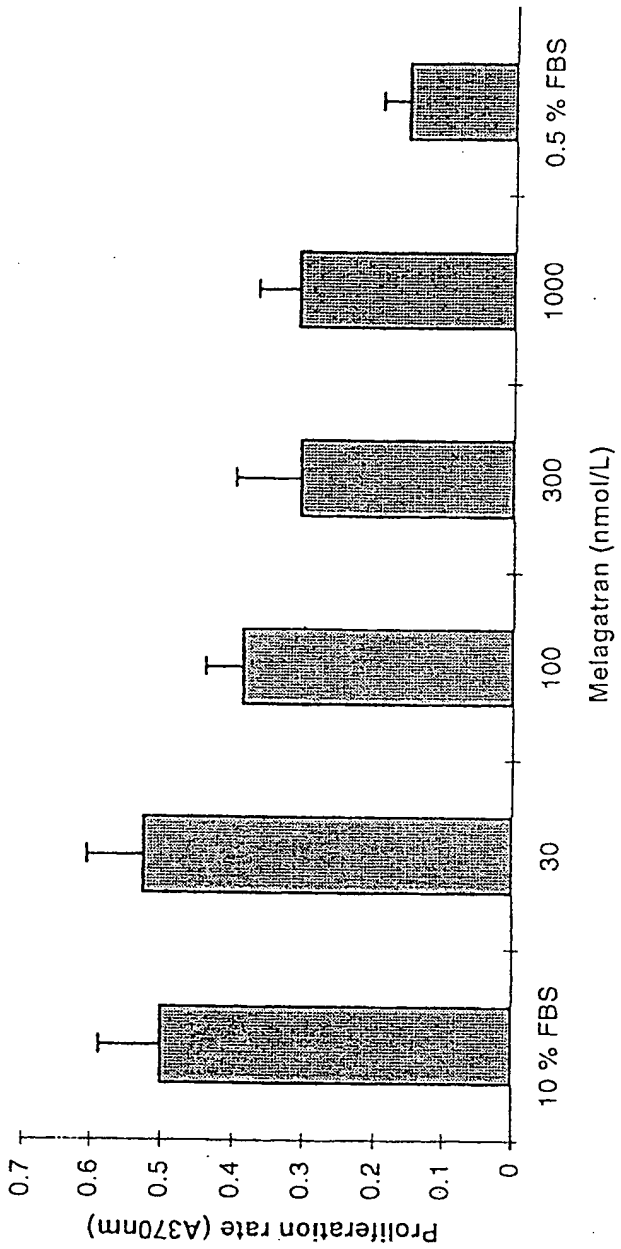

Proliferation rate (A370nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10 % FBS
30
100
300
1000
0.5 % FBS
Melagatran (nmol/L)

Figure 1

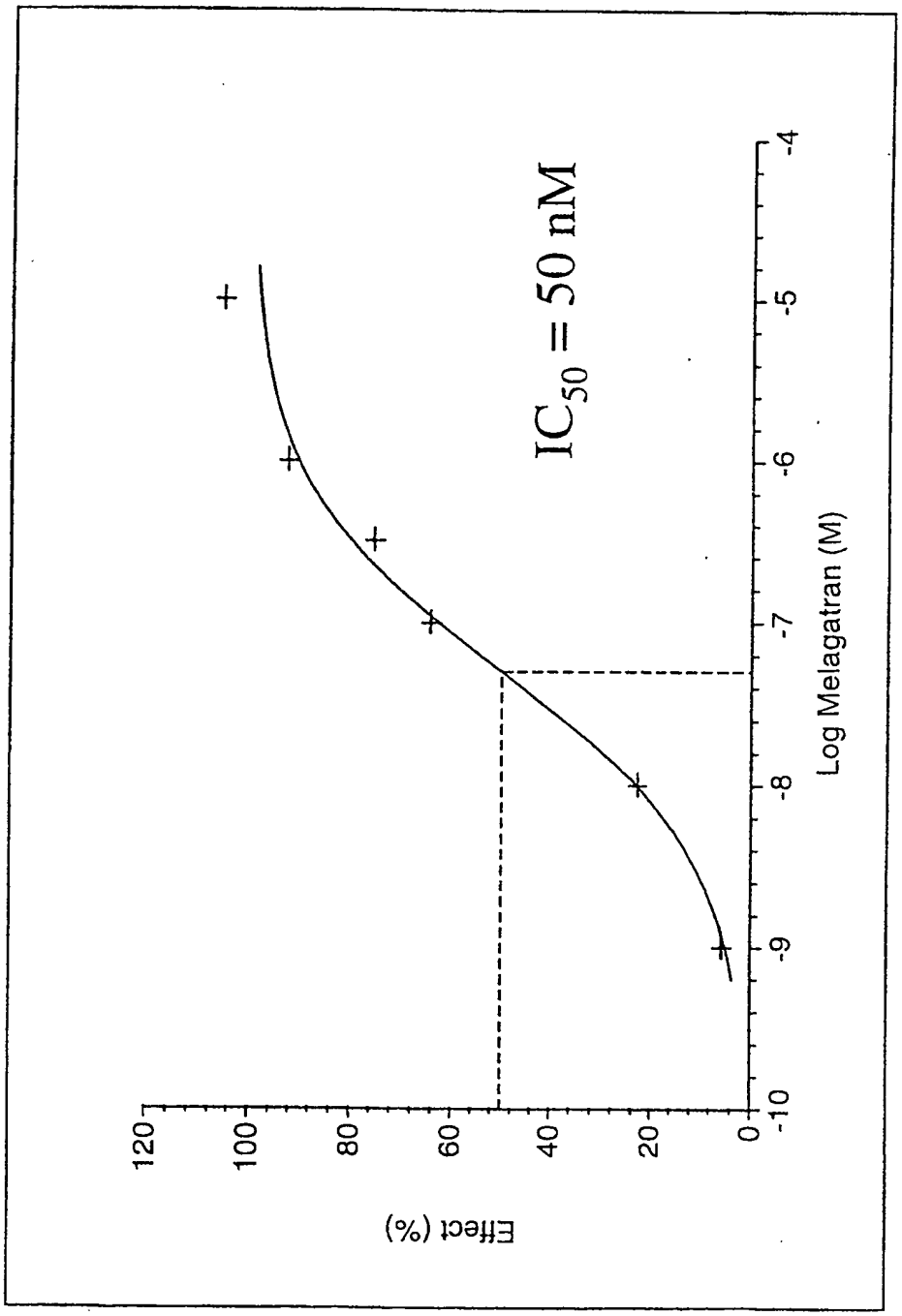

IC_{50} = 50 nM
Effect (%)
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Log Melagatran (M)

Figure 2

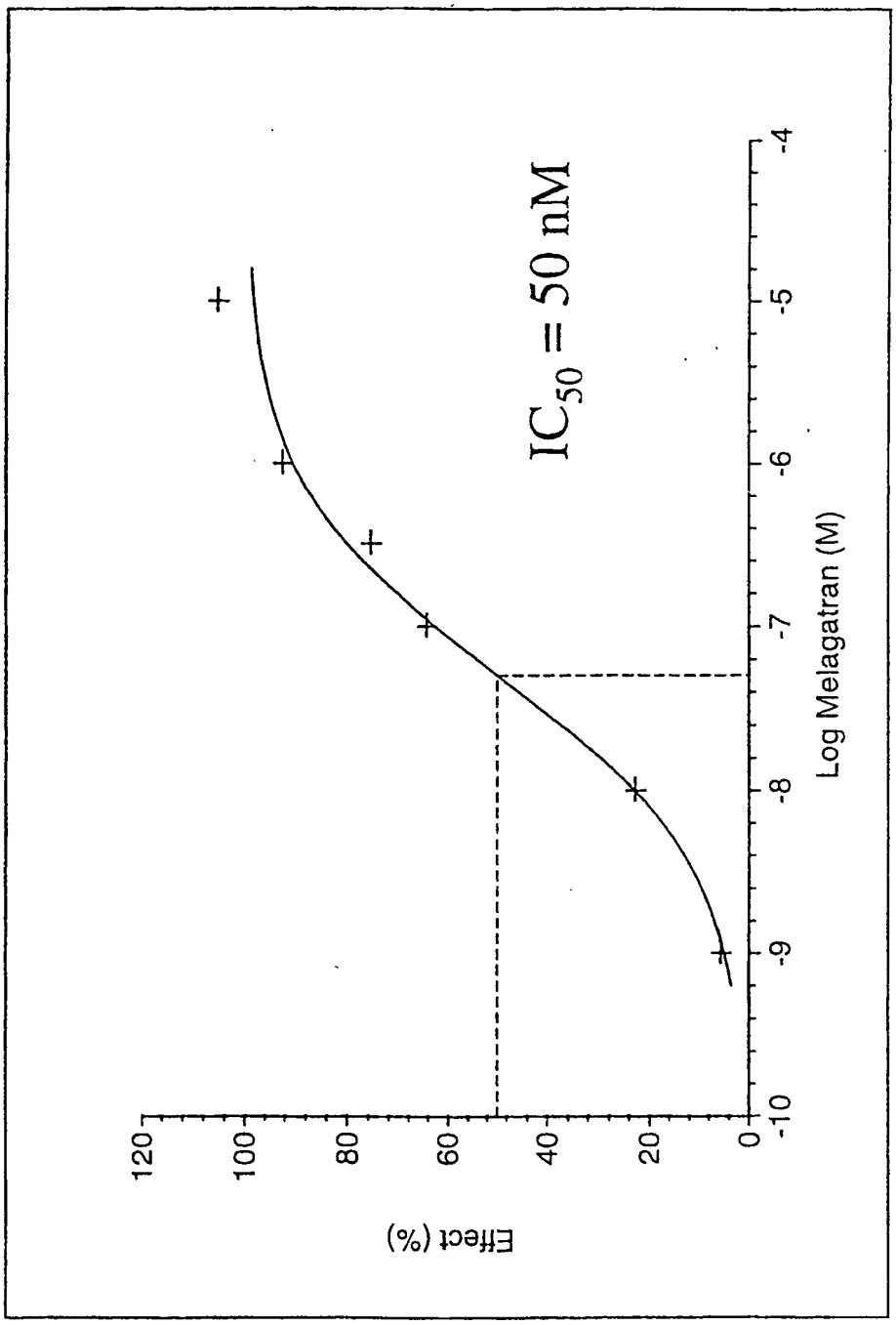

IC_{50} = 50 nM
Effect (%)
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Log Melagatran (M)

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/02433

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 38/55, A61P 35/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the XIth International Symposium on Atherosclerosis, held in Paris, France, on 5-9 October 1997, B. Jacotot et al: "Atherosclerosis XI", 1998, Elsevier --	1-11
X	Journal of the American college of cardiology, Volume 17, No 6, May 1991, Jesse W. Currier et al, "Low Molecular Weight Heparin (Enoxaparin) Reduces Restenosis After Iliac Angioplasty in the Hypercholesterolemic Rabbit" page 118B - page 125B --	1-11
X	EP 0454220 A1 (AKZO N.V.), 30 October 1991 (30.10.91) -- --------	1-11

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2001	03 -04- 2001
Name and mailing address of the ISA: Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/02433

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 38/55, A61P 35/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the XIth International Symposium on Atherosclerosis, held in Paris, France, on 5-9 October 1997, B. Jacotot et al: "Atherosclerosis XI", 1998, Elsevier --	1-11
X	Journal of the American college of cardiology, Volume 17, No 6, May 1991, Jesse W. Currier et al, "Low Molecular Weight Heparin (Enoxaparin) Reduces Restenosis After Iliac Angioplasty in the Hypercholesterolemic Rabbit" page 118B - page 125B --	1-11
X	EP 0454220 A1 (AKZO N.V.), 30 October 1991 (30.10.91) -- --------	1-11

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"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2001	03 -04- 2001
Name and mailing address of the ISA: Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/02433

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-5, 11
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/02433

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-5, 11
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/02433

Claims 1-5, 11 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/02433

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SE 00/02433

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0454220 A1	30/10/91	SE 0454220 T3	
		AT 93235 T	15/09/93
		AU 644429 B	09/12/93
		AU 7525591 A	24/10/91
		BR 1100979 A	07/12/99
		CA 2040905 A	24/10/91
		DE 69100275 D,T	02/12/93
		DK 454220 T	22/11/93
		ES 2060284 T	16/11/94
		FI 101541 B	00/00/00
		FI 911942 A	24/10/91
		HK 1002319 A	00/00/00
		HU 57223 A	28/11/91
		HU 211506 B	28/11/95
		HU 215152 B	28/11/00
		HU 911346 D	00/00/00
		HU 9500312 A	28/09/95
		IE 64378 B	26/07/95
		IE 911277 A	23/10/91
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